

Laboratory Evaluation of Virulence of Heterorhabditid Nematodes to *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae)

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ABSTRACT The Indianmeal moth, *Plodia interpunctella* (Hübner), is a cosmopolitan pest of stored products, infesting most commodities in warehouses and grain bins. We studied the susceptibility of Indianmeal moth adults and larvae to seven entomopathogenic nematode species and strains. The nematodes studied were *Heterorhabditis bacteriophora* Poinar (HP88, Lewiston, and Oswego strains); *H. indica* Poinar, Karunakar, and David (Homl strain); *H. marelatus* Liu and Berry (Point Reyes strain); *H. megidis* Poinar, Jackson, and Klein (UK211 strain); and *H. zealandica* Poinar (NZH3 strain). Overall, the nematodes that had the highest virulence to larvae and adults of Indianmeal moth were *H. indica*, *H. megidis*, and *H. marelatus*. Adult Indianmeal moths seemed to be more susceptible to the nematodes than the larvae, and egg laying was significantly reduced by at least 44% in Indianmeal moths adults that survived exposure to the nematode strains. We conclude that *H. indica*, *H. megidis*, and *H. marelatus* should be studied further as potential biocontrol agents of Indianmeal moth in stored grains and processed commodities.

KEY WORDS biological control, entomopathogenic nematodes, *Heterorhabditis*, Indianmeal moth, stored products

THE INDIANMEAL MOTH, *Plodia interpunctella* (Hübner), is among a cosmopolitan group of stored-product moths in the subfamily Phycitinae (family Pyralidae) that is aggressive in infesting stored products. Indianmeal moths are serious pests because their larvae infest value-added, finished food products that are packaged and ready for retail sale. Female moths lay eggs on or near food packages in response to food odors and also prior larval contamination (Mbata 1987, Phillips and Strand 1994). As larvae emerge from eggs, they tie food particles together with their silk as they feed, and last-stage larvae leave the food and lay down large amounts of silk as they wander in search of pupation sites (Mbata 1985). Although several species of pests (e.g., beetles) are targets for pest control in food facilities, storage moths such as the Indianmeal moth are considered among the most common and visually obvious invaders of pet food, baking mixes, breakfast cereals, and candies made with nuts and chocolate (Platt et al. 1998).

Pest management in postharvest systems in the United States, from raw grain storage to food processing to value-added retail products, is facing a critical situation. Postharvest losses caused by insects in the United States were recently estimated at \$5 billion per year (Pimentel 1991). Chemical insecticides have

been used historically at all facets of the postharvest handling of commodities. Some insecticides are effective and needed in some cases; others are probably ineffective and only add potentially harmful residues to food, which is cause for public concern (Foschi 1989). The Food Quality Protection Act of 1996 (FQPA) calls for a reevaluation of all pesticide labels and is targeting organophosphate insecticides, among which are some residual insecticides (chlorpyrifos methyl, chlorpyrifos ethyl, and malathion) commonly used by the food industry. Methyl bromide, an important fumigant in the management of stored-product insects, is an ozone-depleting substance that falls under an international ban along with other organohalides as mandated by the Montreal Protocol (Bell 1996). In addition to the environmental issues surrounding chemical insecticides is the fact that many populations of stored-product insects are resistant to commonly used chemicals (Georgiou and Saito 1983, Subramanyam and Hagstrum 1995), and consumers continue to demand high-quality food that is also pest and residue free. To overcome the challenges faced by the stored-product industry, alternatives to pesticides are proposed for the management of storage insects.

Entomopathogenic nematodes such as those in the genus *Heterorhabditis* are potent alternatives to chemical insecticides. These nematodes are obligate parasites that kill insects with the aid of mutualistic bacteria (*Photorhabdus* spp.) that inhabit the intestine of the nematode (Poinar 1990, Boemare 2002). The free-living infective juveniles (IJs) enter their host through natural openings such as the mouth, anus, and spi-

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Table 1. Mortality response (95% CI) of fifth-instar Indianmeal moths exposed to selected doses of *H. bacteriophora* HP88

Slope	SE	LD ₂₀	LD ₅₀	LD ₇₅	LD ₉₅
0.7789	0.0105	1.52×10^2 (2.41×10 to 6.23×10^3)	4.76×10^2 (6.56×10 to 1.33×10^3)	1.31×10^4 (3.04×10^3 to 9.62×10^7)	1.58×10^6 (5.30×10^4 to 1.36×10^{16})

n = 20.

racles and release their bacteria that kill the host within 48 h (Poinar 1990). The nematodes generally complete two to three generations before leaving the host insect. These nematodes (harmful to insects but innocuous to mammals including humans) are important biological control agents in the management of several crop insect pests (Grewal and Georgis 1999, Kaya and Gaugler 1993, Shapiro-Ilan et al. 2002). The virulence (disease-causing power) of entomopathogenic nematodes varies among the diverse species and strains (Kaya and Gaugler 1993, Shapiro and McCoy 2000, Shapiro-Ilan 2001a, b). This study investigated the potential of various heterorhabditid nematodes to control the Indianmeal moth. Seven different nematode strains and species were compared for their ability to kill larval and adult stage of Indianmeal moth and how nematode infection affects oviposition in females. Steinernematids were not included in our studies because another group is focusing on that genus (Ramos-Rodriguez et al. 2005).

Materials and Methods

Insects. The culture of *P. interpunctella* was originally obtained from USDA-ARS, Grain Marketing and Research Laboratory, Manhattan, KS, in 2001 and had been reared for nine generations at the Department of Biology, Fort Valley State University, before commencement of this study. The culture was reared on a diet of cornmeal, chick laying mash, chick starter mash, oats, and glycerin (volumetric mixture at 4:2:2: 2:1) and in an environmental chamber set at $28 \pm 1.5^\circ\text{C}$, $70 \pm 5\%$ RH, and a photoperiod of 16:8 (L:D).

Nematodes. The following nematodes were tested for virulence to larvae and adults of *P. interpunctella*: *Heterorhabditis bacteriophora* Poinar (HP88, Lewiston, and Oswego strains); *H. indica* Poinar, Karunakar, and David (Homl strain); *H. marelatus* Liu and Berry (Point Reyes strain); *H. megidis* Poinar, Jackson, and Klein (UK211 strain); and *H. zealandica* Poinar (NZH3 strain). All nematodes were reared in parallel at $\approx 24^\circ\text{C}$ in last-instar greater wax moth, *Galleria mel-*

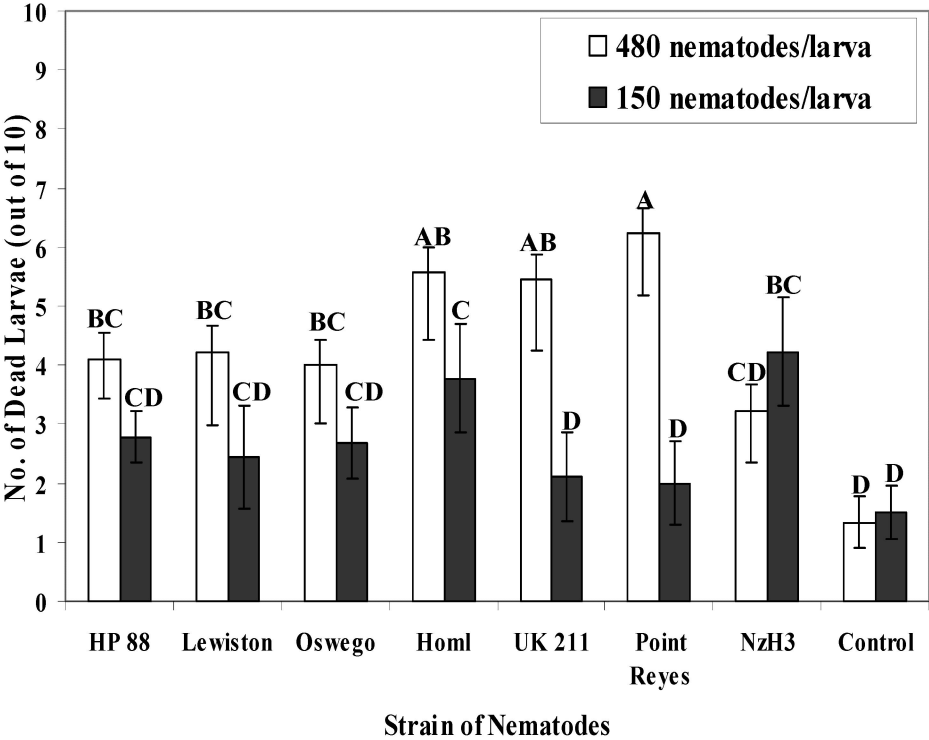


Fig. 1. Mortality of third-instar Indianmeal moths exposed to nematode strains at two doses of infective juveniles (IJ/insect). Different letters above bars indicate statistical significance (Tukey's test, $\alpha = 0.05$).

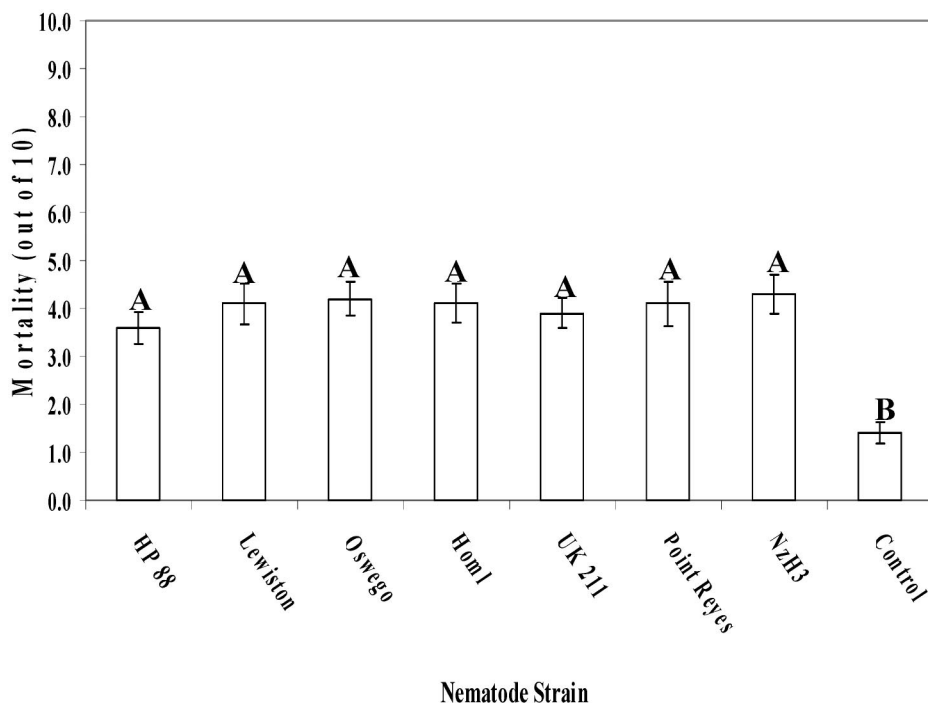


Fig. 2. Mortality (percentage mean \pm SE) of fifth-instar Indianmeal moths exposed to strains of nematodes. Different bars above bars indicate statistical significance (Tukey's test, $\alpha = 0.05$).

lonella L., according to procedures described by Woodring and Kaya (1988). The larvae of *G. mellonella* were obtained from Webster's Waxie Ranch (Webster, WI). Nematodes were stored at 13°C for <8 wk before being used for experiments. Cultures of *H. bacteriophora* (HP88) and *H. megidis* were originally obtained from the MicroBio Group of Becker Underwood (West Sussex, UK), *H. bacteriophora* (Lewiston) and *H. indica* from Integrated BioControl Systems (Lawrenceburg, IN), *H. bacteriophora* (Oswego) from E. Shields (Cornell University, Ithaca, NY), and *H. marelatus* and *H. zealandica* from P. Stock (University of Arizona, Tucson, AZ).

Comparison of Nematode Virulence to Third- and Fifth-Instar Indianmeal Moths. Experiments with larvae of Indianmeal moths were conducted in plastic cups (3–4 cm internal diameter, 3 cm deep; Bioserv, Frenchtown, NJ). Indianmeal moth third- and fifth-instar larvae (10 and 18 d old from egg hatch, respectively) were distributed one per plastic cup containing ≈ 3.5 ml of Indianmeal moth rearing medium. The plastic lids for the cups were replaced on introduction of the Indianmeal moth larvae. The Indianmeal moth larvae in cups containing rearing medium were inoculated with IJs in 0.5 ml of water 1 d after larvae were added and placed in a rearing chamber maintained at $\approx 28 \pm 3^\circ\text{C}$ and $70 \pm 5\%$ RH until adult emergence and assessment of mortality. After the addition of 0.5 ml of water the moisture content of the medium was $\approx 14\%$.

To determine an IJ rate (number/insect) that might be suitable for distinguishing virulence among nematode strains and species, we initially conducted a

dose-response assay with *H. bacteriophora* (HP88 strain). Fifth-instar Indianmeal moths were exposed to 0, 100, 250, 500, 1,000, and 2,000 IJs. Thirty plastic cups were set up for each treatment dose. The infected larvae in cups were incubated in controlled chambers at conditions indicated above for 10 d, at which time Indianmeal moth adult emergence and larval mortality was determined. This experiment was carried out twice. The number of IJ nematodes that caused 20 or 50% larval mortality (LD_{20} or LD_{50}), i.e., 150 or 480 per insect, was used in subsequent assays.

Four replicates of 10 cups per treatment (strain) and an untreated control (water only) were set up for the fifth instars and later for the third instars. Approximately 480 IJs were applied to each cup containing a single Indianmeal moth larva. Larval mortality was recorded after 10 and 21 d for fifth- and third-instar assays, respectively (based on duration to adulthood in chambers maintained at $28 \pm 3^\circ\text{C}$ and $70 \pm 5\%$ RH). Two trials were conducted for each larval stage. When mortality at 480 IJs per Indianmeal moth larva was significantly different among nematodes, susceptibility was also studied at a lower exposure rate of 150 IJs per Indianmeal moths larva.

Comparison of Nematode Virulence to Adult Indianmeal Moths. Indianmeal moth adults used for this study were 12–24 h old at the time of inoculation. Two moist blue filter papers (Anchor Paper Co., St. Paul, MN) were placed on bottom and inner top of the petri dishes (area = 64 cm^2). The blue coloration was chosen to facilitate observance of eggs deposited on the paper. Approximately 50 IJs/ cm^2 (total = 3,200 in 2 ml

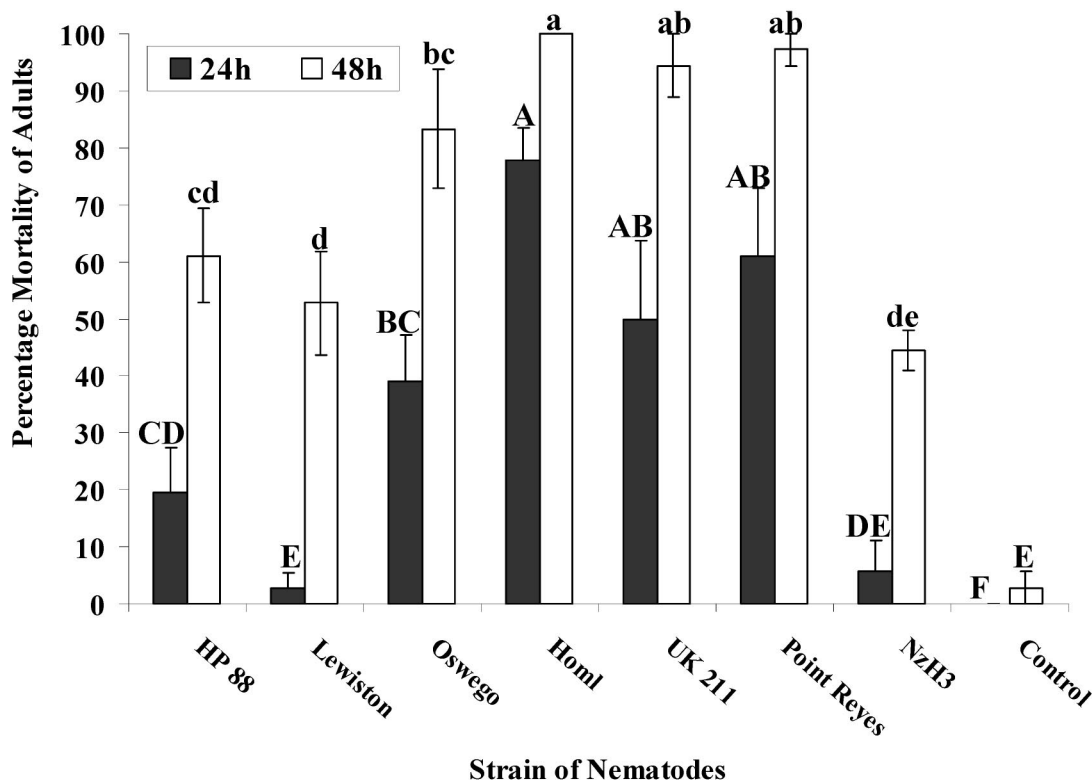


Fig. 3. Mortality (percentage mean \pm SE) of adult Indianmeal moths exposed to strains of nematodes. Different capital letters (24 h) and lowercase letters (48 h) above bars indicate statistical significance (Tukey's test, $\alpha = 0.05$).

of water) were applied onto the filter papers in the petri dishes. Six adults (three males and three females) were placed in each dish. Three dishes were set up for each strain of nematode, and two trials were carried out with two subsequent generations. The control dishes contained the same number of Indianmeal moths, but the filter papers were dispensed with 2 ml of distilled water only. The dishes were kept in chambers maintained at $28 \pm 3^\circ\text{C}$ and $70 \pm 5\%$ RH and inspected for adult Indianmeal moth mortality after 24 and 48 h. The number of eggs laid in each dish was determined after 48 h.

Statistical Analyses. Data generated from experiments with *H. bacteriophora* (HP88) to determine the doses of nematodes that gave 20, 50, 75, and 95% mortality were analyzed with probit analysis by using the PROC PROBIT routine of SAS (SAS Institute 2001). Inputs to the probit analyses were dose (number of nematodes), number of larvae treated, and number that died or failed to complete development to adult. Estimated nematode concentrations to achieve 20, 50, 75, and 95% mortality were compared using the lethal-dose ratio test of Robertson and Preiser (1992).

In the rest of the experiments, treatment effects were determined through analysis of variance (ANOVA; Proc GLM), and if the ANOVA was significant, differences in treatments were elucidated

through Tukey's test ($\alpha = 0.05$) (SAS Institute 2001, Steel and Torrie 1980). Percentage data were arcsine of square root transformed (Steel and Torrie 1980).

Results

Comparison of Nematode Virulence to Third- and Fifth-Instar Indianmeal Moths. A positive dose-response relationship was observed between *H. bacteriophora* (HP88) and fifth-instar Indianmeal moths (Table 1). ANOVA indicated that different strains of nematodes had varying virulence to third-instar larvae of Indianmeal moths at two IJ exposure rates (for 150 IJs/insect: $F = 3.51$; $df = 11$ and 59 ; $P = 0.0008$; for 480 IJs/insect: $F = 27.29$; $df = 11$, 60 ; $P < 0.0001$). Mortality of third-instar larvae was higher at the IJ rate of 480/insect than at 150/insect (Fig. 1). Among the most virulent nematode strains at the rate of 480 IJs/insect were *H. marelatus* (Point Reyes), *H. megidis* (UK211), and *H. indica* (HOM1); *H. marelatus* strain caused greater mortality than all other nematodes except *H. megidis* (UK211) and *H. indica* (HOM1) ($P < 0.05$). At the lower nematode rate (150 IJs/insect), only *H. zealandica* and *H. indica* caused greater mortality than the control (Fig. 1).

Differences in mortality of fifth instars were detected between all treatments and the control (Fig. 2; $F = 6.61$; $df = 12, 67$; $P < 0.0001$). However, segregation

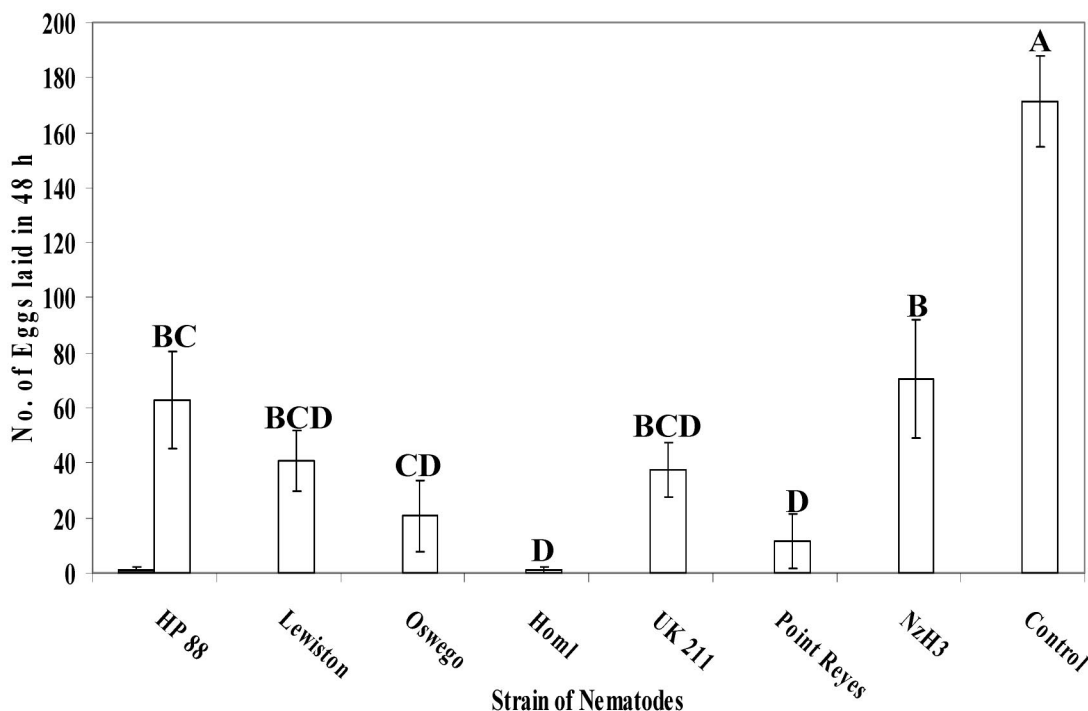


Fig. 4. Effect of infection of Indianmeal moths adults by strains of nematodes on eggs laid within 48 h after exposure. Different bars above bars indicate statistical significance (Tukey's test, $\alpha = 0.05$).

of the means using Tukey's test showed that mortalities were not different among strains, but all nematode strains caused higher mortality than the control.

Comparison of Nematode Virulence to Adult Indianmeal Moths. The effect of nematode strain on mortality of adult moths is shown in Fig. 3. Treatment effects were detected both during the 24-h period of exposure and after an additional 24 h after exposure to nematodes (48 h from the commencement of experiment; at 24 h: $F = 14.19$; $df = 7, 40$; $P < 0.0001$; at 48 h, $F = 28.28$; $df = 7, 40$; $P < 0.0001$). Among the most virulent strains of nematodes to adult Indianmeal moths were *H. indica* (HOM1), *H. marelatus* (Point Reyes), and *H. megidis* (UK211); the *H. indica* strain caused greater mortality than all other nematodes except *H. marelatus*, and *H. megidis* ($P < 0.05$). The least virulent strains were *H. zealandica* (NZH3) and *H. bacteriophora* (Lewiston), which were the only nematodes that did not cause greater mortality than the control after 24-h exposure. Treatment of the adult moths with heterorhabditid nematodes affected the number of eggs laid by female moths within 48 h (Fig. 4; $F = 10.77$, $df = 7$ and 40 ; $P < 0.0001$). The mean number of eggs laid by control females (171.5) was significantly higher than those laid by females exposed to all nematode strains ($P < 0.05$). Egg laying during exposure to *H. indica* (HOM1), *H. marelatus* (Point Reyes), and *H. bacteriophora* (Oswego) was less than the number laid by those exposed to *H. zealandica* (NZH3).

Discussion

Both adults and larval Indianmeal moths were generally most susceptible to *H. marelatus* (Point Reyes), *H. megidis* (UK211), and *H. indica* (HOM1). These nematodes warrant further study for their potential to control Indianmeal moths. Among the nematode strains that possess relatively high virulence to Indianmeal moths are those considered to be cold tolerant, i.e., *H. marelatus* and *H. megidis* (Grewal et al. 1994, Berry et al. 1997), and heat tolerant, i.e., *H. indica* (Shapiro and McCoy 2000). This implies that management of Indianmeal moths with nematodes may be possible year round in certain storage facilities, but with different strains of nematodes.

Although we did not compare the insect stages with each other directly, it seems that Indianmeal moth adults may be more susceptible to heterorhabditids than the larvae. Relative to the larvae, higher mortality was observed in the adults when adult Indianmeal moths were exposed to a similar dose of nematodes per insect but a considerably shorter exposure time. Previous studies have indicated that susceptibility to entomopathogenic nematodes can be affected by insect age or stage (Boivin and Belair 1989, Glazer 1992, Shapiro et al. 1999, Shapiro-Ilan 2001a). For quite a number of insect species, the larvae have been found to be more susceptible than adults (Geden et al. 1985, Fuxa et al. 1988, Glazer 1992, Mannion and Jansson 1992, Morse and Lindegren 1993, Theunis 1998). In support of our hypothesis for Indianmeal moth sus-

ceptibility, however, adult insects have been found to be more susceptible than the larvae in some other insects, e.g., the pecan weevil, *Curculio caryae* (Horn) (Shapiro-Ilán 2001a, b). Conceivably (although we believe less likely), the observed differences in Indianmeal moth larval and adult mortality could be explained by the different media on which the assays were conducted.

Exposure of Indianmeal moth adults to heterorhabditid nematodes significantly reduced oviposition in surviving females. It is interesting to note that strains of nematode, such as *H. zealandica* (NZH3) and *H. bacteriophora* (Lewiston), that did not cause significant mortality in Indianmeal moths adults substantially reduced oviposition. Because virgin adults were exposed to the nematodes, it is possible that mating as well as oviposition was affected by infection of moths by nematodes. The potential impact of heterorhabditid nematodes on oviposition could be a key factor in protecting stored products.

Significant hurdles exist, however, before entomopathogenic nematodes can be employed commercially for Indianmeal moth control. Although laboratory screening of entomopathogenic nematodes for virulence is an important step in developing a biological control program for a given pest (Mannion and Jansson 1992, Stark and Lacey 1999, Shapiro-Ilán et al. 2002), relative virulence among nematodes in laboratory studies may not be consistent with efficacy in the field (Grewal and Georgis 1999). It is necessary to conduct trials in grain stores or warehouses with candidate nematodes that are virulent in the laboratory. In addition, application of nematodes against Indianmeal moths in stored products may have to be carried out by formulating them with attractants into killing stations, because mixing nematodes with commodities may not be acceptable. Thus, the ultimate test of efficacy should be done in warehouses or grain stores by formulating nematodes into killing stations baited with attractants such as pheromones.

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